Figure 1

CAR KNOCK-OUT CONSTRUCT

Mouse cDNA
988 bp

Genomic Structure
of KO Construct

INTRON

47 bp deletion

ATG

pA

ATG

NEO

...tg tg tgg tg tg GGAGACCGGGC

219

CACTAGAAAC c atg g c cagtg...

173

= DNA Binding Domain

Lower Case = Deleted Region

= Neo Insert
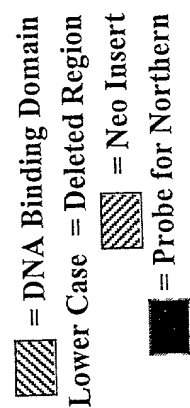
= Probe for Northern

FIGURE 2

Section A.

.....aaaatttacccaacatagatttatctaatgtaattcctatctgcagaacatccaaatactttggaaattatttnttgtggttgtagctgtttgaat

gtaaacatatattcaaaaaaactcttcatggtgatgtagcattgggcaagctatgaggatacctacttctggttatttactaaaagttgatagcca

ggcagtggtggcacacacctttaatcccagcacttgggaggcagaggcaggtggaattatgagtttgaggccagcctggtctacagagtg

ggttcaaggtcagccagggctacacagagaaaccctgtctcaaaaagaaggaggaggaggaggaaagaggaagaggaggaagaaga

tcttttgttttgagatagcatacagtgaaaatttcggtttctttagcaactcagttgtgtcacatgatgtctttctggaagctgtcttgtgagcagaca

tgtgatgtttatcacaatagaaagc.....

Section B.

.....aaagaggtcatcaggcttggcagcaagtgcctttgcctaccgagtctttacaccagctccaccgtggtttttgagacagtctcccactgg

actggatttcagcaagaaagctaggcttgccttcttgtctctgcctccttggcattggaattatgagttgttccaccgtgccattttttaaaaatgta

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caaaaagcaacatttttgctttaatgtcctcagtgctggggagcccggtgtcaggctgggcagtcttgggaagagattctgtagaggagaga

gaagagagtcctatgcccagtgctgattctcaactcctcccacattcaggagacc**ATGACAGCTATGCTAACACTAG**

AAA*CCATGGCCAGTGAAGAAGAATATGGGCCGAGGAA*CTGTGTGGTGTGT*GGAGACC

GGGCCACAGGCTATCATTTCCACGCCCTGACTTGTGAGGGCTGCAAGGGCTTCTTCA

Ggtgaatgcttcctccccaacagaaacaaccccgacatttctatcagtccacctttaaacactggtacacctccaagttataatcctcttgcag

ctaagctgcactgcccagtgtctagcactctcaatcttgctgaccacaacgcagtgtgaaactggtgacctaatgacaaggcaggttaaccat

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gagtaaccttgtgttctcatgaaaacgctccaactacctctgaagacctttgagcacagctcagatgagtctgttgttaaatcgatcc.....

Section C.

.....tgcattgctttctactgaagtgtatcacagatgaatatgagatcgacagaaagtgtgcagggatcccccctgccatctggaaacacttaatt

caatgaagtcccaaggaagcctcagaaactctttcttccttcctccttccttatctggggaggtggagtggccccaactgaagggatggctga

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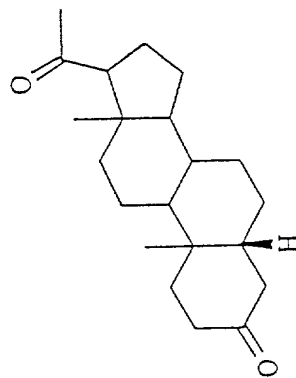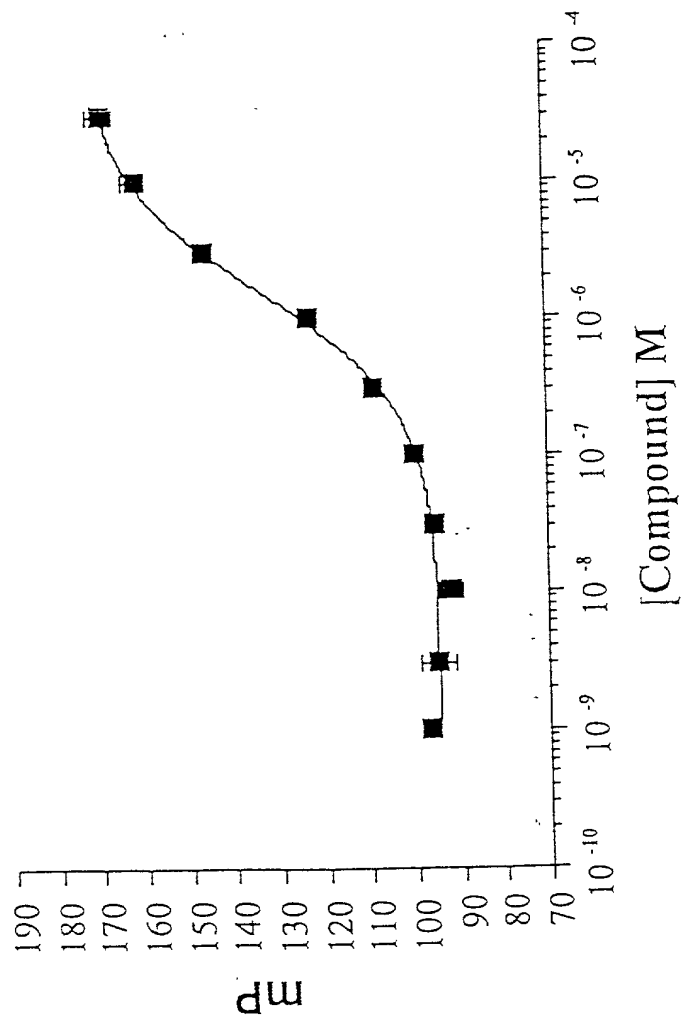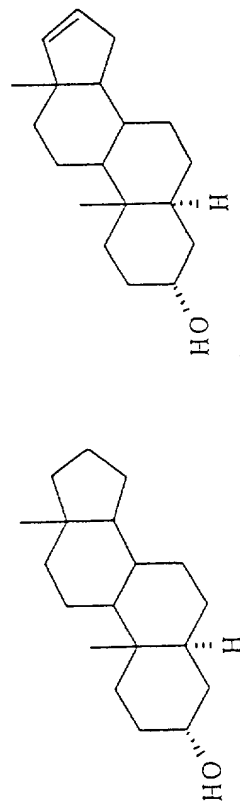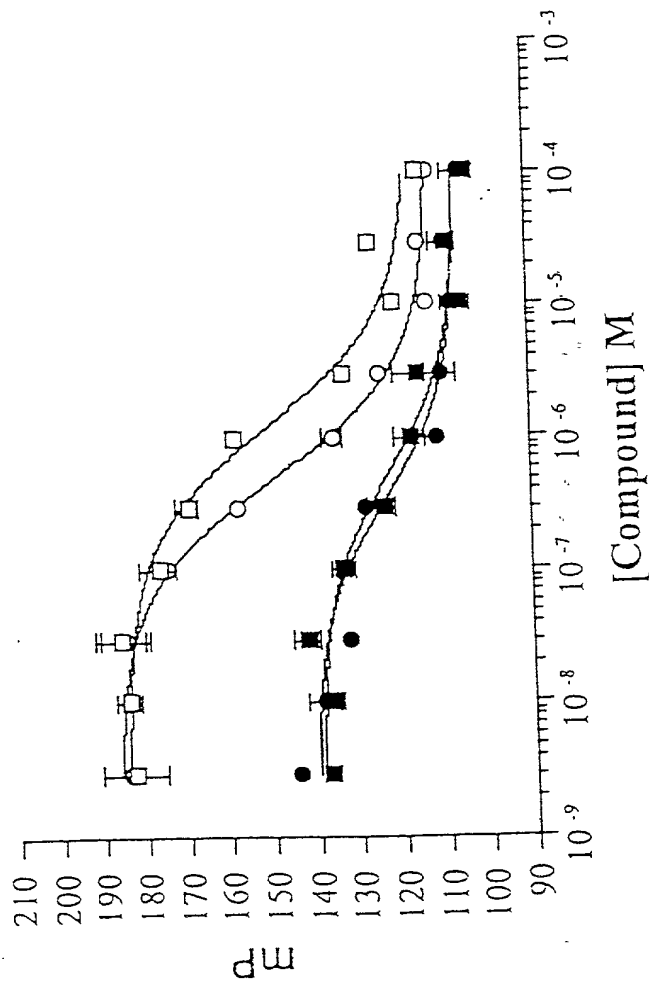
5β-Pregnan-3,20-dione is an
hCAR Agonist

Figure 3

Figure 4

Androstanes are hCAR Antagonists

	IC$_{50}$
□ 5α-Androst-16-en-3 α-ol + 5 μM 5 β-Pregnan-3,20-dione	1.2 μM
○ 5α-Androstan-3 α-ol + 5 μM 5 β-Pregnan-3,20-dione	480 nM
✶ 5α-Androst-16-en-3 α-ol	530 nM
● 5α-Androstan-3 α-ol	340 nM

5α-Androstan-3α-ol

5α-Androst-16-en-3α-ol

FIGURE 5

normalized luciferase activity

ng CAR expression plasmid

☐ vehicle

■ 5α-androstan-3α-ol

▣ 5β-pregnan-3,20-dione

▨ 5α-androstan-3α-ol
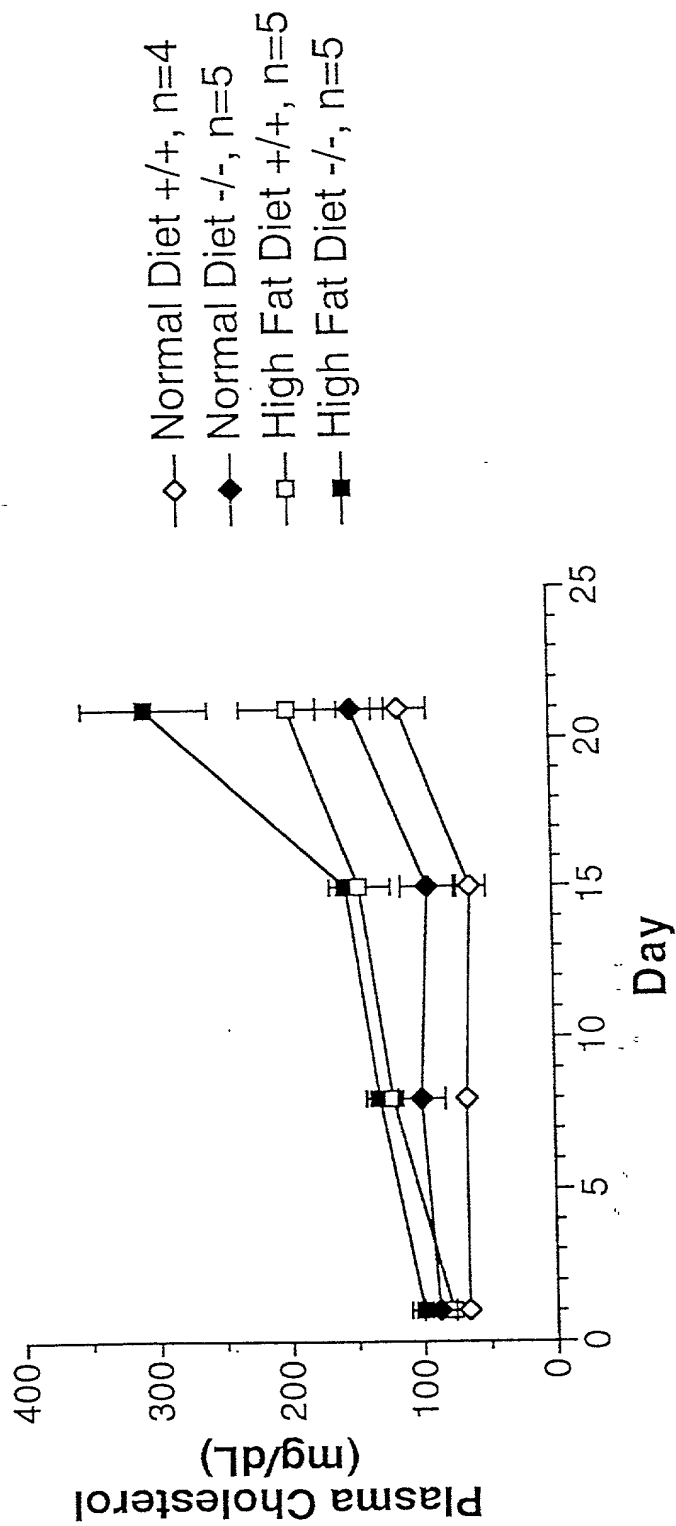+
5β-pregnan-3,20-dione
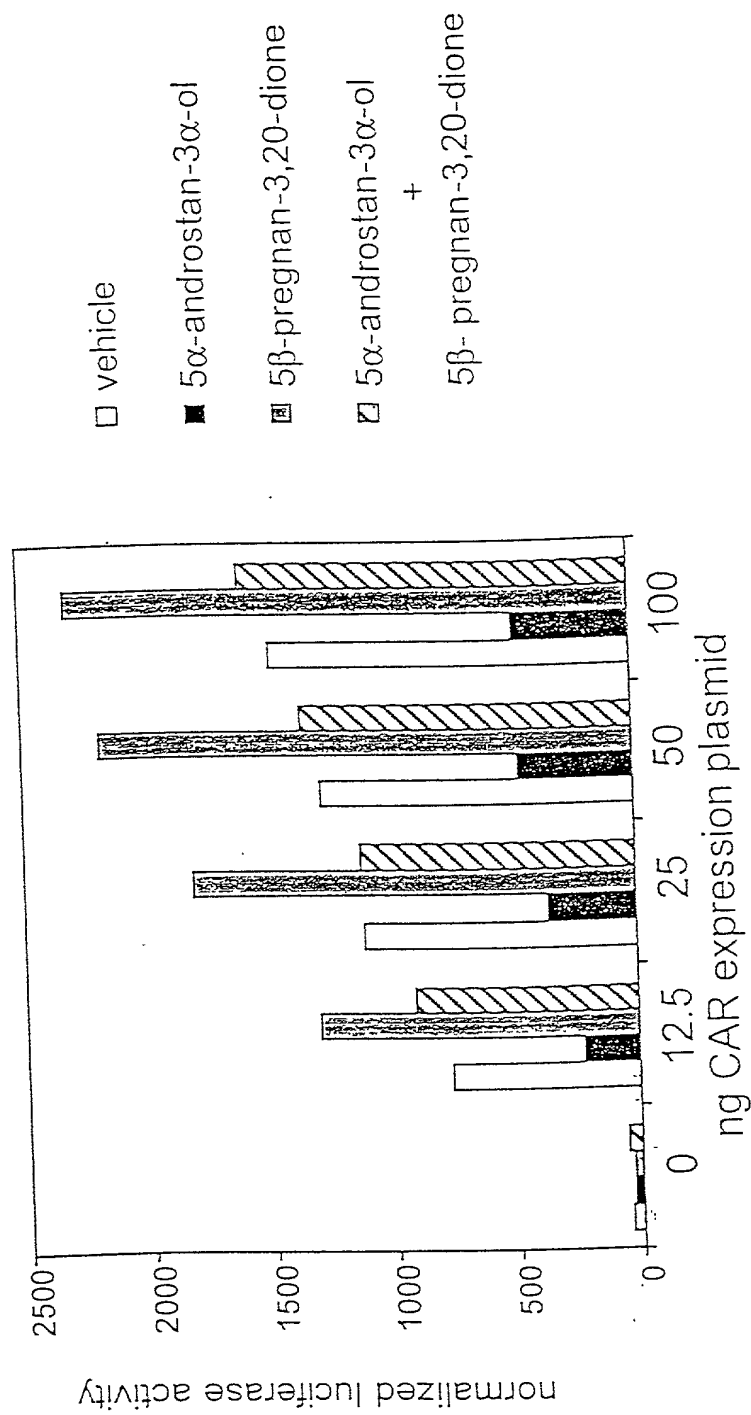
FIGURE 6

Normal diet = Picolab 5053 (containing 4% fat)
High Fat diet = Picolab 5053 (containing 1.25% cholesterol and 10% fat)

FIGURE 7

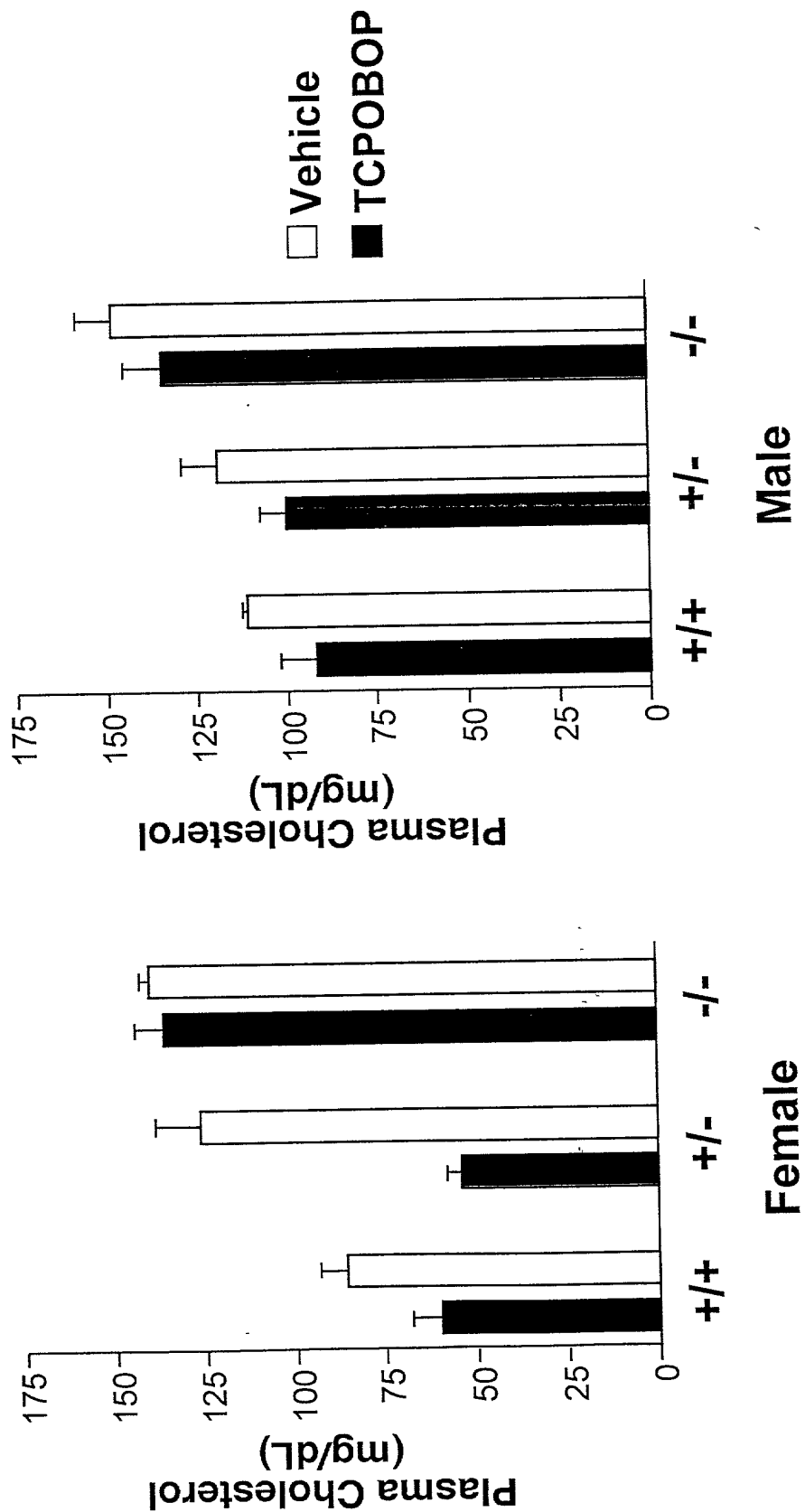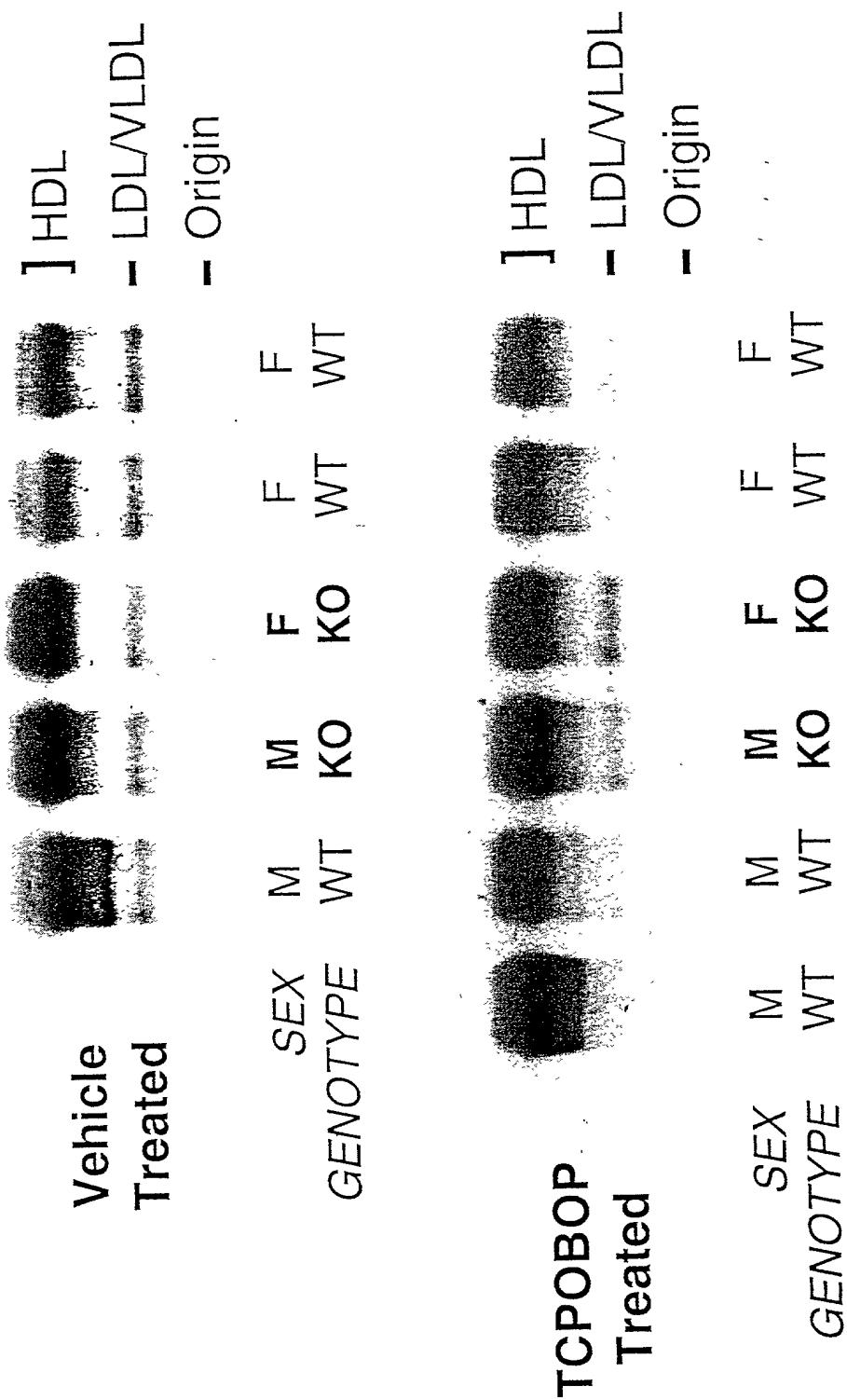FIGURE 8

FIGURE 9